EFFECT OF DIFFERENT THERAPIES ON FERTILITY AND SERUM PROGESTERONE, METABOLITES AND MINERALS PROFILE IN REPEAT BREEDING CROSSBRED COWS

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ABSTRACT

The study was carried out during breeding season on 31 repeat breeding and 8 normal cyclic crossbred cows to evaluate the efficacy of hormonal and antimicrobial drugs in improving fertility and its effect on serum progesterone, metabolites and macro-minerals profile. Among 10, 7 and 7 repeat breeding cows treated with 0.02 mg GnRH i/m just after AI; 500 mg of hydroxy-progesterone caproate i/m on day 4th or 5th post-AI, and antibiotics (Cephalexin or ceftriaxone) i/ut 12-24 hrs post-AI, the conception rates in the treatment cycle were 60.00, 28.57 and 28.57%, respectively, as against only 14.28% in untreated control repeat breeders (n=7) and 62.50% in normal cyclic group. The results of GnRH therapy were better (P<0.05) and at par with the normal fertile group. The average serum P_4 concentration for GnRH treated cows was significantly higher (P<0.05), and at par with that of normal cyclic group, as compared to progesterone treated and untreated control groups. The overall average P_4 concentration of repeat breeders was significantly lower (P<0.05) than that of normal cyclic cows. The circulatory P_4 levels at oestrus were identical and low in most of the groups, but were higher and varied significantly at 20-22 days post-AI due to variable number of animals that conceived in each group. None of the treatments influenced the serum protein profile of repeat breeders. In contrast, the serum cholesterol concentration for GnRH and progesterone treated groups were significantly lower than those of control (247.31±26.50 mg/dl) and normal fertile groups. The mean serum calcium content of repeat breeders and normal fertile cows averaged 8.76±0.25 and 9.66±0.27 mg/dl, respectively; phosphorus 8.90±0.21 and 7.35±0.17 mg/dl, and magnesium 3.70 ±0.15 and 4.16±0.15 mEq/L, all of which differed significantly (P<0.05). The serum inorganic phosphorus content was significantly higher (P<0.05) and magnesium content was lower in GnRH and progesterone treated groups than the antibiotics treated, control and normal cyclic cows. The inorganic phosphorus level was significantly higher in conceived than non-conceived group among repeat breeders. In general, the relatively better results in repeat breeders with GnRH (Busereline), at par with normal fertile cows, suggest that ovulatory problem and endocrine imbalance as the major causes of repeat breeding in cows.

KEY WORDS: Repeat breeding cows, Hormonal/Antibiotics therapy, Fertility, Serum progesterone, Metabolites, Macro-minerals.

INTRODUCTION

Numerous studies have shown that repeat breeding is one of the most prevalent reproductive disorders in dairy cows. For this disorder hormonal and non-hormonal drugs are used by the practitioners. Gonadotropin releasing hormone (GnRH), progesterone and antibiotics have good therapeutic values to enhance fertility/ reproductive efficiency in infertile dairy animals with good nutritional status (Patel *et al.*, 2005[a]; Mahto *et al*., 2006; Singh *et al*., 2008). The variable results reported following these treatments in the repeat breeders may be largely due to ovarian changes, endocrine events, uterine infection and to a greater extent faulty breeding management and semen quality. However, the literature on the influence of these therapies on blood profile of progesterone, metabolites and minerals is scanty (Patel *et al*., 2005[a,b]; Sharma *et al.*, 2008). Hence this study was planned to evaluate the comparative efficacy of GnRH, progesterone and antibiotics therapy on fertility and its influence on serum status of progesterone, metabolites and macro-minerals in repeat breeding crossbred cows under field condition.

MATERIALS AND METHODS

Thirty one individually managed repeat breeding crossbred cows that had taken more than 3 infertile services beyond 6 months postpartum and eight normal cyclic cows formed the material for this study. The repeat

breeding cows were subjected to following therapeutic regimes keeping 7 as untreated control and the results were compared with normal cycle cows.

Group-I : GnRH: Ten repeat breeders were inseminated and simultaneously administered single dose of 0.02 mg GnRH i/m (Inj. Receptal 5 ml).

Group-II : Progesterone: Seven repeat breeding cows were treated with hydroxy progesterone caproate 500 mg i/m (Inj. Duraprogen 2.0 ml) on day 4^{th} or 5^{th} post-AI.

Group-III: Antibiotics: Seven repeat breeding cows were treated either with cephalexin (Lixen, IU) or ceftriaxone (Inj. Vetaceph 2 gm) i/ut 12 to 24 hrs post-AI.

Group-IV : Untreated Control: Seven repeat breeding cows were kept as untreated control.

Group-V : Normal Cyclic /Fertile Control: Eight normal cyclic animals that were presented for first AI within 3 months postpartum served as normal fertile control.

The animals in oestrus were inseminated by the trained inseminators and pregnancy was confirmed per rectum 45 days later. Jugular blood samples were collected on the day of treatment/AI and at 20-22 days post-AI. The serum samples were stored at -20°C till analyzed. Serum progesterone was estimated by RIA technique of Kubasic *et al.* (1984). The sensitivity of the assay was 0.1 ng/ml, and intra- and inter-assay coefficients of variation was 5.4 and 9.1 %. The serum biochemical constituents, viz. total protein, total cholesterol and macro-minerals, viz. calcium, inorganic phosphorus and magnesium were estimated using standard procedures and kits procured from Coral Clinical Systems, Goa, with the help of Chemwell auto-blood analyzer (Awareness Technology, Germany). The conception rates in the treatment cycle were compared between different groups by chi-square test. The data on plasma profile were analyzed by using completely randomized design (Snedecor and Cochran, 1986).

RESULTS AND DISCUSSION

Effect on Fertility

Out of 10, 7 and 7 repeat breeding cows treated with GnRH, progesterone and antibiotics 60.00, 28.57 and 28.57%, respectively, conceived in the treatment cycle itself. The conception rates among 7 untreated control repeat breeders and 8 normal cyclic cows were 14.28 and 62.50 %, respectively. Thus, the conception rate with GnRH therapy in repeat breeders was significantly improved over control and was at par with the normal cyclic cows. The improved conception rate in the treatment group may be attributed to the beneficial effect of GnRH in regulating the time of ovulation and improved fertilization rates in cows together with CL development, progesterone secretion and embryo survival (Dekruif, 1978). The improvement in pregnancy rate after treatment with GnRH at AI may also be related to insufficient GnRH release from hypothalamus causing failure of ovulatory LH surge as its one of the causes. Present findings on comparative merits of different therapies to some extent corroborated with the earlier reports in cows (Patel *et al*., 2005[a]) and buffaloes (Sharma and Dhami, 2008). Further, the results with particular drug over control compared favourably with previous reports on GnRH (Roy *et al*., 1995; Dadarwal *et al.,* 2007), progesterone (Das *et al.*, 2002; Sharma *et al*., 2008) and antibiotics (Mahto *et al*., 2006; Sharma and Dhami, 2008).

Serum Progesterone

The average serum progesterone concentration in repeat breeding cows under different treatment groups including conceived and non-conceived categories was significantly lower at oestrus/AI as compared to that at 20-22 days post-AI. The administration of GnRH immediately after AI in repeat breeders strengthened CL development and progesterone secretion as revealed by significantly higher circulatory P_4 concentration obtained in that group, while exogenous progesterone (Duraprogen) supplementation on day 4-5 post-AI did not appear to give sustainable circulatory progesterone level till day 20-22 post-AI. Moreover, the P_4 value of GnRH treated cows was significantly higher ($P<0.05$), and at par with that of normal cyclic group, as compared to progesterone treated and untreated control groups. Further, the average P_4 concentration of repeat breeders (2.75±0.54 ng/ml) was significantly lower ($P<0.05$) than that of normal cyclic cows (3.87±1.05 ng/ml). Again the circulatory P_4 levels at oestrus were identical and low in most of the groups, but varied

significantly at 20-22 days post-AI due to variable number of animals that conceived in each group. Similarly, conceived cows of both repeat breeding (5.11±1.24 vs 1.45±0.35 ng/ml) and normal cyclic (4.97±1.52 vs 2.03±0.92 ng/ml) groups had significantly higher P_4 as compared to non-conceived cows on day 22 post-AI, though the values at oestrus were identical (Table 1).

The present results of the influence of GnRH and progesterone therapy on serum P_4 profile are in accordance with many previous reports (Patel *et al*., 2005[a]; Sharma *et al.*, 2008). Walton *et al.* (1990) reported that treatment with injectable progesterone (200 mg) on days 5, 7, 9 and 11 post-oestrus did not increase plasma progesterone concentrations over controls in repeat breeding cows. Schmitt *et al.* (1996), however, observed significantly increased plasma P_4 between day 8 to 16 following hCG (3000 IU, i/m) treatment on day 5 post-oestrus, while GnRH at oestrus caused insignificant rise in P_4 in heifers. Ata and Tekin (2001), however, did not find significant variation in plasma P_4 levels at day 14 and 22 after GnRH treatment at oestrus in repeat breeding as compared to control cows. According to Gonzalez *et al*. (1999) GnRH did not stimulate progesterone release in normal cows, but in repeat breeding cows it had a luteotropic effect that delayed regression of CL and maintained high progesterone concentrations. Bage *et al.* (2002) found higher progesterone concentration at the time of AI in non-conceived than conceived repeat breeder animals. In present study also the progesterone concentration at the time of AI was higher in non-conceiving group than in the conceiving group, both in repeat breeder as well as normal cyclic groups.

Serum Total Protein

The serum total protein content of repeat breeders and normal cyclic cows averaged 7.50±0.14 and 7.25±0.19 g/dl, respectively, which did not differ significantly. The total protein content at oestrus and at 22 days post-AI or even the pooled values neither varied significantly between various treatment groups nor between conceived and non-conceived groups (Table 1). Patel *et al*. (2005[b]), however, found significantly higher plasma total protein in hCG treated repeat breeding cows as compared to GnRH and progesterone treated cows, but the values did not vary significantly between conceived and non-conceived cows. Gentile *et al.* (1978) also opined that serum protein level was not related with fertility in dairy cows. Moreover, Tegegne *et al*. (1993) concluded that blood metabolites concentrations were not good indicators of nutritional status and were not related to ovarian activity in cattle. The present findings for group differences corroborated well with the reports of Singh and Pant (1998) and Chandrakar *et al.* (2003). However, Dutta *et al.* (1991) and Mishra *et al.* (2007) reported significantly lower ($P<0.01$) levels of total serum proteins in repeat breeders than the normal cyclic cows.

Serum Total Cholesterol

The serum total cholesterol concentration of repeat breeding cows under different treatment and control group varied significantly ($P<0.05$). The values for GnRH (217.64±19.66 mg/dl) and progesterone (168.36±14.61 mg/dl) treated groups were significantly lower than those of control group (247.31±26.50 mg/dl). The overall pooled total cholesterol content of repeat breeders (215.31±10.66 mg/dl) was, however, significantly lower ($P<0.01$) than that of normal fertile group (279.94±26.33 mg/dl). Moreover, there was no significant variation in total cholesterol levels between oestrus and 22 days post-AI phases in any of the groups, though the values were relatively higher at oestrus than at day 22 post-AI in normal fertile group (310.74±25.22 vs 249.11±45.44 mg/dl). Similarly, the cholesterol concentration at both the phases was higher in non-conceived than the conceived cows of normal fertile group, but did not differ significantly (Table 2). Patel *et al.* (2005[b]) reported significantly lower serum cholesterol in GnRH treated group as compared to hCG and progesterone treated groups of repeat breeding cows and even control cows, but not between conceived and non-conceived cows. Singh and Pant (1998) also recorded the plasma levels of total cholesterol to be significantly higher in normal cyclic than the repeat breeding cows (240.90±11.68 vs 125.49±4.03 mg/dl), but the values were much lower than the present ones. Srivastava and Sahni (2000) observed the levels of cholesterol at oestrus/ AI in cows turned out to be pregnant and non-pregnant as 80.29±3.11 and 67.34±1.93 mg %, respectively, which is significantly inversed than the present observation. The wide variations observed in blood cholesterol values between different studies may be attributed to breed, age, reproductive status, nutritional status, health, body condition of animals and even assay technique employed.

Serum Calcium

The overall average serum calcium content of repeat breeders and normal fertile cows averaged 8.76±0.25 and 9.66±0.27 mg/dl, respectively, which differed significantly (P<0.05). The pooled values at oestrus were insignificantly higher than those at 22 days post-AI in both repeat breeders and normal fertile group. Further, the calcium value of conceived normal fertile cows was apparently higher than the non-conceived cows, though no such trend was observed in repeat breeders. It was almost consistent at around 8 mg/dl in repeat breeders and around 9 mg/dl in normal fertile animals (Table 2). Patel *et al.* (2005[b]) recorded significantly lower serum calcium in hCG treated repeat breeding cows as compared to GnRH treated cows, which is in contrast to the present observations. The present findings with respect to trend between repeat breeders and cyclic cows, however, are in agreement with many previous reports (Kalita *et al.,* 1999; Dutta *et al.,* 2001). Although Chandrakar *et al.* (2003) reported significantly lower (P<0.01) levels of serum calcium in normal fertile crossbred cows (6.17±0.17 mg/dl) as compared to repeat breeding cows (9.63±0.36 mg/dl). However, Singh and Pant (1998) and Das *et al.* (2002) reported identical serum calcium level in normal cyclic and repeat breeding cows under rural condition.

Serum Inorganic Phosphorus

The average serum inorganic phosphorus content of repeat breeders was significantly (P<0.05) higher than in normal fertile cows (8.90±0.21 vs 7.35±0.17 mg/dl). Further, the serum inorganic phosphorus value of conceived normal fertile cows was little lower than the non-conceived cows, while reverse trend was observed in repeat breeders with significantly higher values in conceived than non-conceived group (9.66±0.36 vs 8.48±0.22 mg/dl). In general, the serum phosphorus content was significantly higher (P<0.05) in GnRH and progesterone treated groups than the antibiotics treated, control and normal cyclic cows. It was non-significantly higher at 20-22 days post-AI than at oestrus in all the treatment groups, control group and even normal cyclic group (Table 3). Patel *et al.* (2005[b]) found significantly higher serum inorganic phosphorus in both hCG and GnRH treated group as compared to progesterone treated group of repeat breeding cows and even control cows. Lotthmer *et al*. (1974) reported that deficiency as well as excess levels of phosphorus impaired fertility. Agarwal *et al.* (1985) reported identical mean inorganic phosphorus levels in repeat breeding and normal cyclic cows (8.03±0.82 and 8.19±1.33 mg/dl). Significantly lower blood inorganic phosphorus levels in repeat breeders as compared to normal cyclic crossbred cows have also been reported earlier (Singh and Pant, 1998; Dutta *et al.,* 2001; Das *et al*., 2002).

Serum Magnesium

The serum magnesium concentration of repeat breeding cows under different treatment and control groups varied significantly (P<0.05). The values for GnRH and progesterone treated groups were significantly lower than that of control group as well as antibiotic treated group. The overall mean magnesium content of repeat breeders (3.70±0.15 mEq/L) was also significantly lower (P<0.05) than that of normal fertile group (4.16±0.15 mEq/L). An increasing trend was observed in magnesium concentration in all treatment groups from oestrus to day 20-22 post-AI, while in control group and normal cyclic animals, the values had decreasing trend (Table 3). The magnesium concentration at both oestrus and 22 days post-AI was apparently higher in non-conceived than the conceived group of treated repeat breeders, while in normal cyclic animals, it was reverse. Kalita et al. (1999), however, reported the mean concentration of magnesium to be significantly (P<0.05) higher in normal cyclic (3.57±0.15 mg %) than in the repeat breeders (2.74±0.18 mg %) as well as anoestrus (2.58±0.20 mg %) cows. Dutta et al. (2001) reported significantly higher (P<0.01) mean magnesium level in cyclic (3.19±0.07 mg %) than in repeat breeding (2.56±0.12 mg %) cows. However, no report was available to compare the effect of different treatments observed on magnesium profile in repeat breeding cows under study. Significantly lower magnesium levels in hormone treated cows could be due to increased activity of certain enzyme systems involving magnesium.

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